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13. ABSTRACT (Maximum 200 Words)
We have studied whether chronic alcohol exposure alters expression and/or function of hippocampal kainite receptors (KA-Rs). Under the support of this award, we demonstrated that interneuronal KA-Rs are among the most ethanol sensitive receptors in the central nervous system (see attached reprints #1 and 2). Based on these results and those of a previous study with CA3 pyramidal neurons, we expected KA-Rs to be upregulated in response to long-term ethanol exposure. During the last year, we have used the ethanol inhalation route to test this possibility. Unexpectedly, we found that 24 h withdrawal from long term exposure to ethanol vapor produced only small non-significant changes in KA-R subunit levels. Radioligand binding studies also yielded unexpected results. Specifically, [^{3}H]-vinylidene KA binding was unaffected in the CA3 region, dentate gyrus and cerebellum but significantly decreased in the colliculi, entorrhinal cortex and pre-frontal cortex. Taken together, these results indicate that hippocampal KA-R are minimally affected by long-term ethanol exposure and suggest that these receptors could be involved in the maintenance of sensitivity to ethanol. During the last year of support (non-cost extension), we will concentrate on characterizing the effect of ethanol on the function of presynaptic KA-Rs in the CA3 region.

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INTRODUCTION:

As with other drugs of abuse, long-term alcohol ingestion results in the development of tolerance, addiction, and dependence. Alcohol produces these effects by altering the actions of neurotransmitters and their receptors in the brain. A group of proteins affected by chronic ethanol exposure are ligand-gated ion channels such as the glutamatergic ionotropic receptors. Glutamate activates three major classes of ionotropic receptors. These three major types of channels are the NMDA, AMPA and kainate receptors (KA-Rs). The purpose of this proposal is to test whether or not chronic ethanol exposure results in alterations in subunit expression and/or function of KA-Rs in the hippocampus. We have also extended our studies to the acute effects of ethanol on KA-Rs on different neuronal subpopulations within hippocampus.

BODY:

Our overall strategy is to perform studies that assess KA-R expression and function in parallel. Western blot, radioligand binding and immunohistochemical experiments are being used to determine the effects of chronic ethanol exposure and withdrawal on the expression levels of these receptors. Patch-clamp electrophysiological experiments with hippocampal slices are being used to determine the functional consequences of chronic exposure to ethanol and withdrawal. Our specific objectives are:

Objective #1: To determine whether chronic ethanol exposure results in a change in expression of KA-Rs. To measure [^{3}H] vinylidene-kainate binding to hippocampal tissue sections from control, chronically ethanol-treated rats and ethanol-withdrawn rats. To measure levels of GluR5, GluR6/7 and KA2 subunits in hippocampal by using Western blot and immunohistochemical techniques.

Objective #2: To determine whether chronic ethanol exposure results in changes in the function of pre- and postsynaptic KA-Rs in rat hippocampal CA1 and CA3 pyramidal neurons. We are using whole cell patch-clamp electrophysiological methods to determine the effects of chronic ethanol exposure and withdrawal on synaptic and agonist-evoked kainate currents. We are also measuring effects on presynaptic kainate receptor-mediated inhibition of evoked excitatory and inhibitory synaptic currents in rat hippocampal CA1 and CA3 pyramidal neurons.

The following **Statement of Work** was proposed to complete these objectives:

Year #1:

We will perform Western blot and immunohistochemistry experiments, quantify, and interpret the results of these experiments. We estimate that we will be able to complete experiments with anti-GluR6/7 antibodies during the first year. We will also initiate the electrophysiological characterization of kainate receptor-mediated synaptic and evoked currents in the CA3 region of the hippocampus. We will present our preliminary findings at a scientific meeting.

Year #2:

We will continue Western blot and immunohistochemistry experiments, quantify, and interpret the results of these experiments. We estimate that we will be able to complete experiments with anti-GluR6/7 antibodies and initiate studies with anti-GluR5 antibodies during the second year. We will complete the electrophysiological characterization of kainate receptor-mediated synaptic and evoked currents in the CA3 region of the hippocampus. We will initiate the electrophysiological characterization of kainate receptor function in the CA1 region of the hippocampus, including experiments on kainate receptor-mediated regulation of GABA release. We will present our preliminary findings at a scientific meeting.

Year #3:

We will finish Western blot and immunohistochemistry experiments, quantify, and interpret the results of these experiments. We will complete experiments with anti-GluR5 antibodies and with anti-KA2 antibodies during the last year. We will also complete electrophysiological experiments of kainate receptor function in the CA1 region of the hippocampus, including experiments on kainate receptor-mediated regulation of GABA release. We will present our preliminary findings at a scientific meeting. We will submit a paper to a peer-review scientific journal reporting the findings of our study.

Research Accomplishments:

Western Blot Studies:

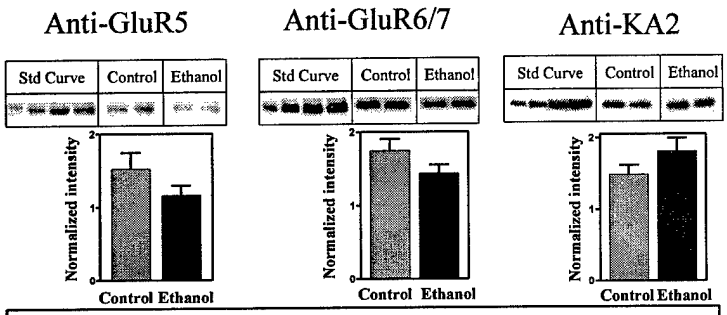

Fig 1. Withdrawal from long-term ethanol vapor exposure minimally affects KA-R subunit expression levels as determined by Western immunoblotting assays; n = 8.

A series of Western blot experiments were completed in Year #1. Findings of these studies were published in Ferreira et al., Ionotropic glutamate receptor subunit expression in the rat hippocampus: lack of an effect of a long-term ethanol exposure paradigm. *Alcohol Clin Exp Res* 25, 1536-1541, 2001. These studies were performed with tissue from rats exposed to an ethanol-containing liquid diet that produced blood alcohol levels ranging between 20-50 mM. During Year #3, we performed similar studies with tissue from rats exposed to ethanol vapor for 2 weeks (blood alcohol levels between 50-75 mM) and then withdrawn for 24 hr. Unexpectedly, expression of GluR5, GluR6/7 and KA2 subunits was not significantly affected (Fig 1; $p > 0.2$ by t-test). These results confirm that long-term ethanol exposure minimally affects KA-R subunit expression.

Receptor Autoradiography:

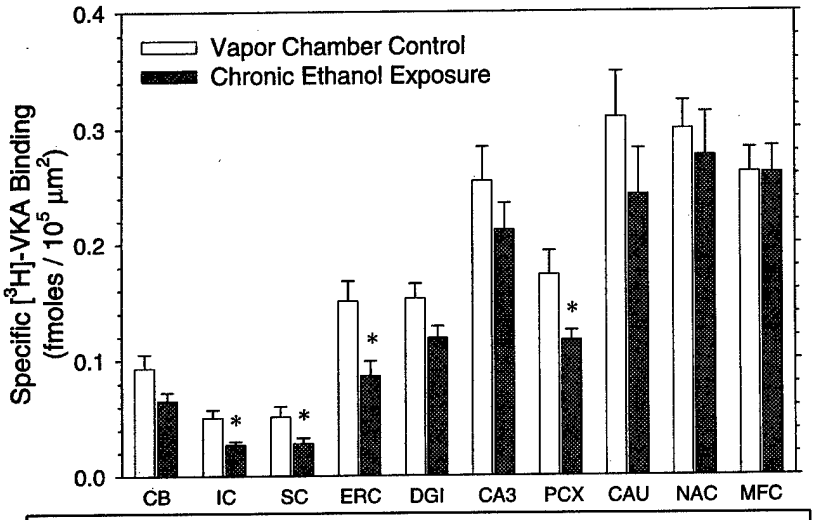

Fig 2. Withdrawal from long-term ethanol vapor exposure does not significantly affect hippocampal [3H]-VKA binding but significantly affect several extrahippocampal regions. *P<0.05 by t-test; n = 7.

During Year #2, we completed the analyses of confocal images acquired from brain sections from rats exposed to a liquid diet containing ethanol and pair-fed controls. These studies were discussed in the previous report and revealed that the chronic ethanol diet did not affect expression levels for GluR6/7 kainate receptor subunits. During Year #3 we measured [^{3}H]-vinylidene-kainate ([^{3}H]-VKA) binding on brain sections from rats withdrawn from the 2-week vapor chamber exposure (Fig 2). Surprisingly, we found that [^{3}H]-VKA binding in the CA3 region, dentate gyrus (DGI), cerebellum (CB), caudate (CAU), nucleus accumbens (NAC) and medial frontal cortex (MFC) was unaffected and that it was significantly reduced in the inferior colliculi (IC), superior colliculi (SC), entorhinal cortex (ERC) and prefrontal cortex (PCX). Together with our published finding that KA-Rs in the CA3 region are potently inhibited by acute ethanol exposure (Weiner et al., *Mol. Pharmacol.* 56:85-90, 1999), these results confirm that hippocampal KA-Rs do not undergo maladaptive up-regulation in response to the inhibitory actions of ethanol.

Slice electrophysiological studies:

During Years #1-3, we originally proposed to initiate studies of kainate receptor function in the CA3 region and to initiate experiments in the CA1 region. During the last two years, we have actually focused on characterizing the effects of ethanol on CA1 kainate receptors. Experiments on CA3 kainate receptors will be performed in Year #4. Prior to performing electrophysiological experiments with slices from animals chronically-exposed to ethanol, we had to characterize the acute effects of ethanol on these receptors. We concentrated our efforts on interneuronal kainate receptors, since this population of receptors is the only one that is activated by synaptic release of glutamate in the CA1 region (Cossart et al., 1998; Frerking et al., 1998).

Data collected in Dr. Weiner's laboratory during Years #1-2 demonstrated that ethanol potently inhibited KA-R-mediated inhibition of $GABA_A$ synaptic responses recorded from CA1 pyramidal neurons in rat brain slices. The results from these studies have been already discussed in the Sept 2002 report and are now published (see reprint #2). Dr. Weiner also studied the effects of chronic ethanol exposure on interneuronal kainate receptors at GABAergic synapses in the rat hippocampus. The results of these studies were also discussed in the previous report. Briefly, these studies revealed that although rats maintained on the liquid diet achieved blood ethanol levels of approximately 50 mM

(~0.25 g/dl), chronic ethanol exposure had no effect on the KA-R-dependent modulation of $GABA_A$ IPSC amplitude. In addition, the acute potentiating effect of ethanol on $GABA_A$ IPSCs was also not affected by this treatment protocol. Moreover, identical results were observed in slices prepared from rats that had been withdrawn from ethanol for 24 hours. As mentioned in the previous report, work performed in both the Valenzuela and Weiner laboratories provided more direct evidence of a potent effect of ethanol on interneuronal kainate receptors (see reprint #1 in appendix). Together, the results of the studies reported in the attached papers suggest that although presynaptic kainate receptors at GABAergic synapses in the rat hippocampus are extremely sensitive to acute ethanol exposure, no adaptation to this effect occurs following repeated exposure to ethanol. The fact that the magnitude of these acute synaptic interactions are not diminished following repeated ethanol exposure suggests that these effects may play a significant role in the synaptic mechanisms associated with repeated and excessive ethanol consumption. A manuscript reporting the results of these studies is currently in preparation.

Plans for Year 4:

A 1-year non-cost extension has been approved to perform additional studies on the acute effects of ethanol on presynaptic kainate receptors in the CA3 region of the hippocampus. In this region, KA-Rs regulate glutamate release and long-term potentiation. Given our previous results with postsynaptic KA-Rs in this region and in CA1 interneurons, we anticipate that ethanol will also potently modulate pre-synaptic CA3 kainate receptors.

KEY RESEARCH ACCOMPLISHMENTS (YEAR 3):

- Western immunoblotting studies with hippocampal homogenates from control rats and rats exposed to ethanol vapor for 2 weeks followed by 24 h withdrawal. These studies revealed minimal effects on GluR5, GluR6/7 and KA2 levels. Autoradiography with [^{3}H]-VKA revealed that hippocampal KA-R density is also unaffected by this treatment. However, we detected significant decreases in [^{3}H]-VKA binding in extrahippocampal brain regions.
- Completed electrophysiological characterization of the effects of acute ethanol exposure on presynaptic KA-Rs at inhibitory GABAergic synapses in CA1 region of the hippocampus (see attached reprints #1 and 2). A manuscript reporting the electrophysiological characterization of the effect of the 16-day liquid diet and withdrawal on the function of these receptors is currently in preparation.

REPORTABLE OUTCOMES (YEAR 3):

Peer-reviewed articles (see appendix):

Crowder, T.L., Ariwodola, O.J., and **Weiner, J.L.** Ethanol antagonizes kainate receptor-mediated inhibition of evoked $GABA_A$ IPSCs in the rat hippocampal CA1 region. *J Pharmacol Exp Ther.* 303:937-44, 2002.

Carta, M., Ariwodola, O.J., Weiner, J.L., and **Valenzuela, C.F.** Alcohol potently inhibits the kainate receptor-dependent excitatory drive of hippocampal interneurons. *Proc. Natl. Acad. Sci. USA.* 100, 6813-6818, 2003.

Meeting Presentations:

Valenzuela, C.F., Non-linear Effects of EtOH on Interneuronal Kainate Receptors in the CA1 Hippocampal Region. Oral presentation at the Research Society for Alcoholism meeting, Ft. Lauderdale, Fl, June 21-June 26, 2003.

Carta, M., Dettmer, T.S., **Valenzuela, C.F.** Effects of ethanol on interneuronal kainate receptors in the CA1 hippocampal region. Poster presentation at the Society for Neurosciences meeting, Orlando, Fl, Oct 2-7, 2002.

Ariwodola, O.J. and **Weiner, J.L.** Effect of chronic ethanol on kainate receptor function in the rat hippocampus. Poster presentation at the Annual Meeting of the Society for Neuroscience, Orlando, 2002.

Crowder, T.L. and **Weiner, J.L.** Ethanol Inhibits Presynaptic Kainate Receptor Function at GABAergic Synapses in the Rat Nucleus Accumbens Core. Poster presentation at the Annual Meeting of the Society for Neuroscience, Orlando, 2002.

CONCLUSIONS:

We have established that chronic ethanol exposure via liquid diet or inhalation does not affect kainate receptor expression. Moreover, the function of kainate receptors in CA1 interneurons is also unaffected. Withdrawal itself does not have an effect either. However, we have conclusively demonstrated that CA1 interneuronal KA-Rs are among the most sensitive receptors to ethanol in the brain. We will now focus our attention to the characterization of ethanol's effects on presynaptic CA3 receptors.

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Ethanol Antagonizes Kainate Receptor-Mediated Inhibition of Evoked $GABA_A$ Inhibitory Postsynaptic Currents in the Rat Hippocampal CA1 Region

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ABSTRACT

Many studies have demonstrated that ethanol reduces glutamatergic synaptic transmission primarily by inhibiting the *N*-methyl-D-aspartate subtype of glutamate receptor. In contrast, the other two subtypes of ionotropic glutamate receptor (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate) have generally been shown to be insensitive to intoxicating concentrations of ethanol. However, we have previously identified a population of kainate receptors that mediate slow excitatory postsynaptic currents in the rat hippocampal CA3 pyramidal cell region that is potently inhibited by low concentrations of ethanol. In this study, we examined the effect of ethanol on kainate receptor-mediated inhibition of evoked $GABA_A$ inhibitory postsynaptic currents (IPSCs) in the rat hippocampal CA1 pyramidal cell region. Under our recording conditions, bath application of 1 μM kainate significantly inhibited $GABA_A$ IPSCs. This inhibition seemed to be mediated by the activation of somatodendritic kainate receptors on GABAergic interneurons and the subsequent activation of metabotropic $GABA_B$ receptors, because the kainate inhibition was largely blocked by pretreating slices with a $GABA_B$ receptor antagonist. Ethanol pretreatment significantly antagonized the inhibitory effect of kainate on $GABA_A$ IPSCs, at concentrations as low as 20 mM. In contrast, ethanol did not block the direct inhibitory effect of a $GABA_B$ receptor agonist on $GABA_A$ IPSCs. The results of this study suggest that modest concentrations of ethanol may antagonize presynaptic, as well as postsynaptic, kainate receptor function in the rat hippocampus.

Alcoholism represents an imposing medical and socioeconomic concern for our society (Volpicelli, 2001). Surprisingly, little is known about the physiological factors that predispose an individual to this disease or the molecular mechanisms that mediate the intoxicating actions of ethanol. Recent studies have suggested that ethanol acts primarily by modulating the activity of a select group of neurotransmitter systems that mediate excitatory and inhibitory synaptic transmission (Faingold et al., 1998; Tsai and Coyle, 1998). It is thought that the summation of these multiple synaptic effects of ethanol underlies the complex behavioral sequelae associated with the intoxicating and reinforcing actions of this drug, and ultimately, the addiction process.

This research was supported by National Institutes of Health Grants AA12251 and AA11997, the Alcoholic Beverage Medical Research Foundation, and U.S. Army Grant DAMD17-00-1-0579.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

DOI: 10.1124/jpet.102.038471.

The majority of excitatory synaptic communication in the mammalian central nervous system (CNS) is mediated by the neurotransmitter glutamate. Glutamate activates three major classes of ionotropic receptors, named for the ligands α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), kainite (KA), and *N*-methyl-D-aspartate (NMDA) (Mayer and Westbrook, 1987). Given the central role that glutamate receptors play in numerous aspects of normal brain function, many studies have examined ethanol effects on glutamatergic synaptic transmission. To that end, there is now compelling evidence, from behavioral, neurochemical, and electrophysiological studies, that ethanol potently inhibits the activity of the NMDA subtype of glutamate receptor and that this inhibition contributes, in part, to some of the behavioral and cognitive effects of this drug (Deitrich et al., 1989; Tsai and Coyle, 1998; Woodward, 2000). In contrast, most studies have reported little or no effect of ethanol on glutamatergic responses mediated by non-NMDA (AMPA and kainate) receptors (Lovinger et al., 1990;

ABBREVIATIONS: CNS, central nervous system; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; KA, kainate; NMDA, *N*-methyl-D-aspartate; IPSC, inhibitory postsynaptic current; EPSC, excitatory postsynaptic current; eIPSC, evoked inhibitory postsynaptic current; aCSF, artificial cerebrospinal fluid; QX-314, *N*-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium chloride; LY 303070, (−)-1-(4-aminophenyl)-3-methylcarbamoyl-4-methyl-7,8-methylenedioxy-5*H*-2,3,-benzodiazepine; IPSC, inhibitory postsynaptic current; APV, DL-(−)-2-amino-5-phosphonovaleric acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline; DNQX, 6,7-dinitroquinoxaline-2,3-dione; SCH 50911, (−)-(*R*)-5,5-dimethylmorpholinyl-2-acetic acid ethyl ester HCl.

Martin et al., 1991; but see Nie et al., 1994; Martin et al., 1995; Valenzuela et al., 1998a).

Interestingly, in many of these previous studies, it was not possible to distinguish between AMPA and kainate receptor-mediated responses. With the relatively recent development of selective AMPA receptor antagonists (Paternain et al., 1995), it is now apparent that AMPA receptors are the primary mediators of fast excitation at most non-NMDA receptor-gated synapses. Thus, in many previous reports of ethanol-insensitive non-NMDA receptors, responses were likely mediated predominantly by AMPA receptors.

The physiological role of the kainate subtype of glutamate receptor is only now beginning to emerge and little is known about the pharmacological properties of native kainate receptors. Although kainate receptors are widely expressed in the CNS, functional kainate receptor-gated synapses have only been identified in a limited number of brain regions (for reviews, see Chittajallu et al., 1999; Frerking and Nicoll, 2000; Lerma et al., 2001). However, in addition to their somewhat limited postsynaptic role, functional presynaptic kainate receptors have been identified in a variety of brain areas. Activation of presynaptic kainate receptors has been shown to potently modulate neurotransmitter release in several brain regions, for example, the hippocampus (Chittajallu et al., 1996; Cossart et al., 1998; Frerking et al., 1999) and the striatum (Chergui et al., 2000; Crowder and Weiner, 2002).

We recently demonstrated that at least one population of kainate receptors in the rat hippocampus is sensitive to low concentrations of ethanol (Weiner et al., 1999). Ethanol, at concentrations as low as 20 mM, significantly inhibited kainate EPSCs recorded from rat hippocampal CA3 pyramidal neurons. In contrast, AMPA EPSCs in this brain region were insensitive to ethanol, even at the highest concentration tested (80 mM). These findings suggest that kainate receptors may represent a novel neuronal target of ethanol action in the mammalian CNS.

In the present study, we sought to determine whether another kainate receptor-mediated response within the hippocampus might also be inhibited by intoxicating concentrations of ethanol. We evaluated the effect of ethanol on kainate receptor-mediated inhibition of evoked $GABA_A$ IPSCs (eIPSCs) in the rat hippocampal CA1 region. Recent evidence suggests that this effect is mediated by the activation of somatodendritic kainate receptors on presynaptic GABAergic interneurons (Cossart et al., 1998; Frerking et al., 1998) and that the subunit composition of these receptors may differ from that of the postsynaptic receptors underlying kainate EPSCs onto CA3 pyramidal neurons (Mulle et al., 2000). Our data suggest that ethanol, at concentrations similar to those that inhibit postsynaptic kainate receptors in the CA3 region, also inhibits kainate receptor-mediated inhibition of eIPSCs onto rat hippocampal CA1 pyramidal neurons. These results further support the hypothesis that native kainate receptors are significantly inhibited by relatively modest concentrations of ethanol and may potentially mediate some of the behavioral and cognitive effects of this drug.

Materials and Methods

Hippocampal Slice Preparation. Transverse hippocampal slices (400 μm) were prepared from 4- to 6-week-old male Sprague-Dawley rats as described previously (Weiner et al., 1997). Slices were incubated at ambient temperature (20–23°C) for ≥2 h before recording in artificial cerebrospinal fluid (aCSF) containing 126 mM NaCl, 3 mM KCl, 1.5 mM $MgCl_2$, 2.4 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 11 mM glucose, and 26 mM $NaHCO_3$, saturated with 95% O_2, 5% CO_2.

Electrophysiological Recordings. Slices were transferred to a recording chamber maintained at 20–23°C and superfused with aerated aCSF at 2 ml/min. Patch electrodes were prepared from filamented borosilicate glass capillary tubes (inner diameter 0.86 mm) using a horizontal micropipette puller (P-97; Sutter, Novato, CA). Electrodes were filled with a recording solution containing 130 mM KGlu, 15 mM KCl, 0.1 mM $CaCl_2$, 1.0 mM EGTA, and 2 mM Mg-ATP (Sigma-Aldrich, St. Louis, MO), 0.2 mM Tris-GTP (Sigma-Aldrich), 10 mM HEPES, and 5 mM QX-314 (pH adjusted with KOH; 275–285 mOsM). Reagents used in the preparation of the recording solution were purchased from Fluka (Buchs, Switzerland) unless otherwise indicated. Whole-cell patch-clamp recordings were made from individual CA1 pyramidal neurons voltage-clamped at −45 to −55 mV. Only cells with a stable access resistance of 5 to 20 MΩ were used in these experiments. Whole-cell currents were acquired using an Axoclamp 2B or Axopatch 200B amplifier, digitized (Digidata 1200B; Axon Instruments, Union City, CA), and analyzed on- and off-line using an IBM compatible PC computer and pClamp 8.0 software (Axon Instruments).

Pharmacological Isolation of IPSCs. Evoked $GABA_A$ receptor-mediated inhibitory postsynaptic currents were evoked every 20 s by electrical stimulation (0.2-ms duration) using a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) placed near the CA1 pyramidal cell body region ("proximal" stimulation; Weiner et al., 1997). Unless otherwise indicated, eIPSCs were pharmacologically isolated using a cocktail of 50 μM APV to block NMDA receptors and either 10 μM LY 303070 (generous gift from Eli Lilly & Co., Indianapolis, IN) or 1 μM NBQX to block AMPA receptor function. QX-314 (5 mM; Alamone Laboratories, Jerusalem, Israel) was included in the patch-pipette solution to block $GABA_B$ IPSCs. Unless otherwise stated, all drugs used were purchased from Sigma-Aldrich. A 4 M ethanol solution (Aaper Alcohol and Chemical, Shelbyville, KY), diluted in deionized water, was prepared immediately before each experiment from a 100% stock solution kept in a glass storage bottle. All drugs were applied directly to the aCSF via calibrated syringe pumps (Razel, Stanford, CT).

Statistics. All drug effects were quantified as the percentage of change in IPSC amplitude relative to the mean of control and washout values. Statistical analyses of drug effects were performed using the two-tailed Student's paired t test or a one-way analysis of variance followed by the Newman-Keuls post hoc test with a minimal level of significance of $P < 0.05$.

Results

Effect of Kainate on eIPSCs. We first examined the effects of exogenous kainate application on the amplitude of pharmacologically isolated eIPSCs recorded from rat hippocampal CA1 pyramidal cells. Neurons were voltage-clamped at depolarized potentials (−45 to −55 mV) and eIPSCs were evoked every 20 s in the presence of the NMDA receptor antagonist APV (50 μM) and the noncompetitive AMPA receptor antagonist LY303070 (10 μM). We have previously shown that these concentrations of APV and LY303070 completely block NMDA and AMPA EPSCs, but have no significant effect on kainate receptor function in rat hippocampal neurons (Weiner et al., 1999). Synaptic currents evoked under these recording conditions were mediated solely by the activation of $GABA_A$ receptors because they were completely antagonized by bath application of the selective $GABA_A$ receptor antagonist bicuculline methiodide (data not shown). A 5- to 7-min bath application of 1 μM kainate significantly inhibited

the amplitude of eIPSCs in all cells tested (to 34.3 ± 3.3% of control, $n = 11$, $P < 0.01$) (Fig. 1, A and D). The onset of this inhibition was rapid and persisted for the duration of the kainate application. The effect was fully reversible upon washout with recovery taking between 20 to 45 min. Under these recording conditions, the inhibition of eIPSCs by 1 μM kainate was not accompanied by a significant change in holding current or input resistance.

It has been reported in murine studies that low concentrations of NBQX can be used to selectively antagonize AMPA receptor function (Bureau et al., 1999; Mulle et al., 2000). We therefore determined whether a low concentration of NBQX was selective for AMPA over kainate receptors in the rat hippocampal CA1 region. Bath application of 1 μM NBQX completely blocked AMPA EPSCs (by 97.8 ± 2.9%; $n = 4$; data not shown) and notably, 1 μM kainate had the same inhibitory effect on $GABA_A$ IPSCs regardless of whether 1 μM NBQX (38.5 ± 3.1% of control, $n = 10$, $P < 0.01$) or 10 μM LY303070 was used to antagonize AMPA receptor activity (Fig. 1, B and D).

We next sought to demonstrate that the inhibitory effect of kainate on eIPSCs required the activation of kainate receptors. Because selective kainate receptor antagonists are not commercially available, we used a protocol in which eIPSCs were first pharmacologically isolated using a blocker cocktail containing maximally effective concentrations of NMDA and AMPA receptor antagonists (either APV + LY303070 or APV + NBQX). Slices were then perfused with a high concentration of the mixed AMPA/kainate (KA) receptor antagonist DNQX and subsequently challenged with 1 μM kainate. In the presence of the blocker cocktail, bath application of 80 μM DNQX had no effect on the amplitude of eIPSCs (Fig. 1C), suggesting that, under our recording conditions, there was no tonic kainate receptor-dependent regulation of GABAergic synaptic transmission. However, DNQX pretreatment completely blocked the inhibitory effect of exogenous kainate application on the amplitude of eIPSCs (94.7 ± 4.5% of control, $n = 6$, $P > 0.05$) (Fig. 1, C and D).

Effect of Ethanol on Kainate Modulation of eIPSCs. We next examined the effect of ethanol on kainate inhibition of eIPSCs recorded in the presence of the AMPA and NMDA receptor antagonist blocker cocktail. Bath application of 80 mM ethanol significantly increased the amplitude and area of eIPSCs, as we have reported previously (Fig. 2A) (Weiner et al., 1997). After a 10-min pretreatment in 80 mM ethanol, slices were then challenged with 1 μM kainate in the contin-

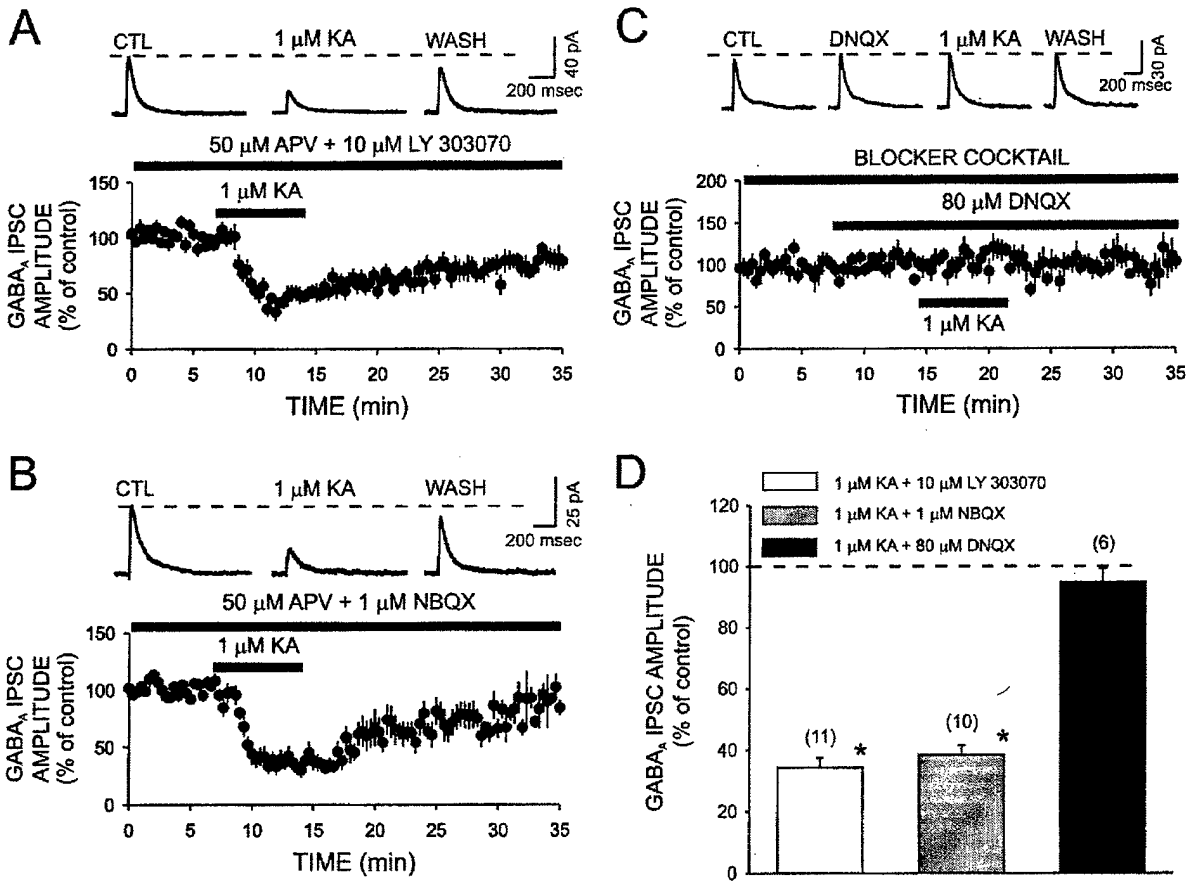

Fig. 1. Activation of kainate receptors inhibits eIPSCs in rat hippocampal CA1 pyramidal neurons. Summary time courses (6–11 cells) of the effect of a 5- to 7-min bath application of 1 μM KA on the amplitude of eIPSCs pharmacologically isolated with 50 μM APV and 10 μM LY303070 (A) or 50 μM APV and 1 μM NBQX (B). The summary time course in C illustrates that bath application of 1 μM kainate has no effect on eIPSCs in the presence of a maximal concentration of the mixed AMPA/kainate receptor antagonist DNQX. Traces above each graph are averages of five to eight eIPSCs recorded under the conditions indicated. D, bar graph summarizing the effect of bath application of 1 μM kainate on the amplitude of eIPSCs recorded in the presence of the selective AMPA receptor antagonists LY303070 and NBQX, and the mixed AMPA/kainate receptor antagonist DNQX. All recordings were carried out in the presence of 50 μM APV. ⋆, significant difference relative to control, $P < 0.05$. Numbers in parentheses indicate the number of cells tested under each experimental condition.

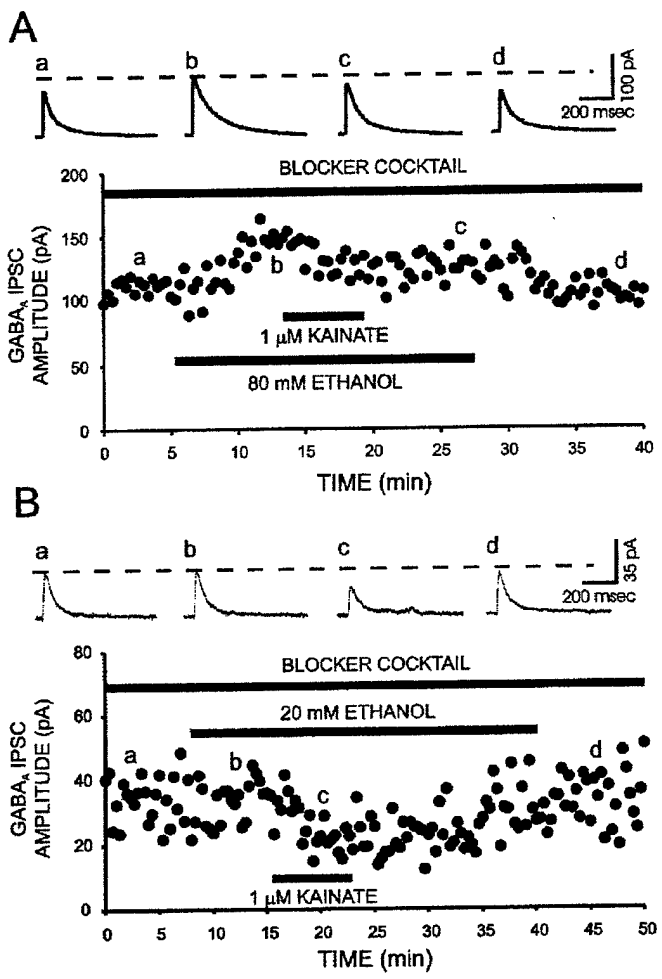

Fig. 2. Effect of ethanol on kainate inhibition of eIPSCs. Time course illustrating the effect of 80 mM (A) and 20 mM (B) ethanol on the inhibitory effect of 1 μM kainate on eIPSCs recorded from rat hippocampal CA1 pyramidal neurons. Traces above the graph are averages of five to eight eIPSCs recorded at the times indicated by the letters.

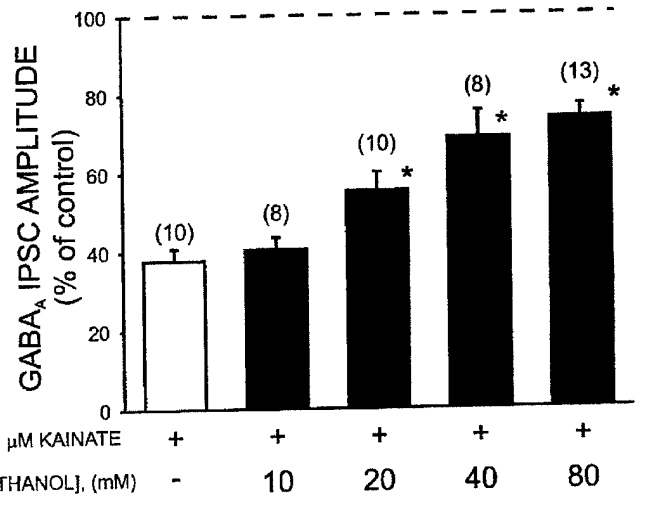

Fig. 3. Concentration dependence of ethanol antagonism of kainate inhibition of eIPSCs. $\star$, $P < 0.05$, relative to the effect of 1 μM kainate alone. Numbers in brackets indicate the number of cells recorded under each experimental condition.

ued presence of ethanol. Although kainate did significantly inhibit the amplitude of eIPSCs in the presence of 80 mM ethanol (to 73.6 ± 3.3% of control, $n = 13$, $P < 0.05$), the magnitude of this inhibition was significantly less than that observed in the absence of ethanol ($P < 0.01$) (Fig. 3). We next examined the concentration dependence of the ethanol antagonism of kainate-mediated inhibition of eIPSCs. Ethanol pretreatment produced a concentration dependent reduction of kainate-mediated inhibition of eIPSCs, with a significant effect being observed at 20 mM ethanol (to 55.3 ± 4.8% of control, $n = 10$, $P < 0.05$), a concentration that had no effect on the amplitude or area of $GABA_A$ IPSCs under these recording conditions (Figs. 2B and 3).

Mechanism of Kainate Inhibition of eIPSCs. A number of mechanisms have been described to account for the inhibitory effect of kainate on eIPSCs in the rat hippocampus (for reviews, see Chittajallu et al., 1999; Frerking and Nicoll, 2000; Ben-Ari and Cossart, 2000; Lerma et al., 2001). A recent study demonstrated that the inhibitory effect of a relatively high concentration of kainate (10 μM) on eIPSCs in the rat CA1 region could be blocked to a significant extent by pretreating slices with a $GABA_B$ receptor antagonist (Frerking et al., 1999). The authors concluded that kainate activates somatodendritic kainate receptors on presynaptic GABAergic interneurons, resulting in a large increase in spontaneous GABA release. This increased GABA release in turn activates presynaptic $GABA_B$ receptors that are known to produce a pronounced decrease in evoked GABA release (Davies et al., 1990), thereby contributing to the kainate-mediated decrease in eIPSCs. To determine whether a similar mechanism was responsible for the inhibitory effect of a lower concentration of KA, we tested the effect of 1 μM kainate on eIPSCs in the presence of the $GABA_B$ receptor antagonist SCH 50911. Under our recording conditions, bath application of 20 μM SCH 50911 dramatically reduced the inhibitory effect of 1 μM kainate on eIPSCs. In fact, kainate had no significant effect on eIPSCs in the presence of SCH 50911, reducing eIPSC amplitude to only 83.2 ± 6.7% of control ($n = 11$, $P < 0.08$). This experiment suggests that the majority of the kainate inhibition of eIPSCs observed under our recording conditions is likely due to presynaptic kainate receptor-dependent release of GABA and the subsequent activation of presynaptic $GABA_B$ receptors (Fig. 4).

Effect of Ethanol on $GABA_B$ Receptor-Mediated Inhibition of eIPSCs. The preceding experiment suggested that the inhibitory effect of 1 μM kainate on $GABA_A$ IPSCs was triggered by the activation of somatodendritic KA receptors on presynaptic GABAergic interneurons but also involved the secondary activation of presynaptic $GABA_B$ receptors. We therefore sought to determine whether ethanol was acting to inhibit the function of these interneuronal KA receptors or, perhaps, was acting downstream to antagonize presynaptic $GABA_B$ receptor function. To differentiate between these two possible mechanisms, we directly assessed the effect of ethanol on presynaptic $GABA_B$ receptor-mediated inhibition of $GABA_A$ IPSCs. Under our recording conditions, bath application of 2.5 μM baclofen, a selective $GABA_B$ receptor agonist, significantly inhibited the amplitude of $GABA_A$ IPSCs (to 42.2 ± 6.1% of control, $n = 8$, $P < 0.001$) (Fig. 5A). This inhibition was completely blocked by pretreating slices with the $GABA_B$ receptor antagonist SCH 50911 (Fig. 5, A and C), suggesting that baclofen inhibition of $GABA_A$ IPSCs was mediated by the activation of $GABA_B$

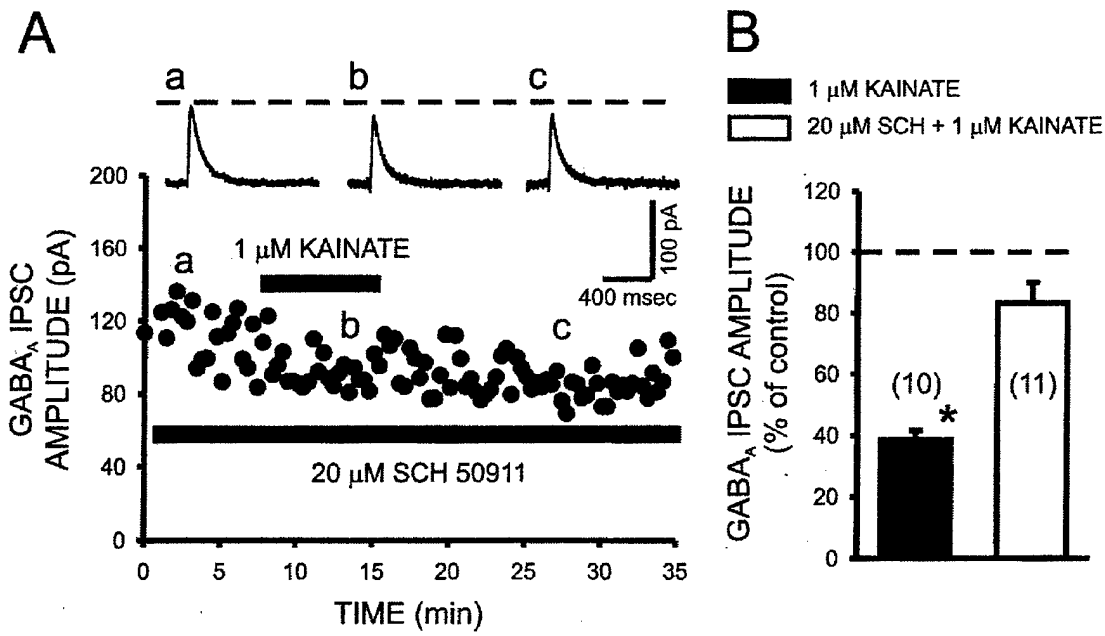

Fig. 4. Blockade of kainate inhibition of $GABA_A$ IPSCs by the selective $GABA_B$ receptor antagonist SCH50911. A, time course illustrating that 1 μM kainate has no effect on the amplitude of eIPSCs in the presence of 20 μM SCH50911. Traces above the graph are averages of five to six eIPSCs recorded at the times indicated by the letters. B, bar graph summarizing the effect 1 μM kainate on the amplitude of eIPSCs in the absence and presence of 20 μM SCH50911. *, $P < 0.05$, relative to control. Numbers in parentheses indicate the number of cells recorded under each experimental condition.

receptors. We next tested the effect of 2.5 μM baclofen in the presence of ethanol. As observed above, pretreating slices with 80 mM ethanol significantly potentiated $GABA_A$ IPSCs. However, ethanol pretreatment did not block the inhibitory effect of baclofen on $GABA_A$ IPSCs (Fig. 5B). In fact, the inhibitory effect of 2.5 μM baclofen was modestly enhanced in the presence of 80 mM ethanol (to 27.0 ± 3.8% of control, $n = 9$, $P < 0.01$) (Fig. 5C).

Discussion

Previous work from our laboratory has demonstrated that relatively low concentrations of ethanol significantly inhibit postsynaptic kainate receptor function in rat hippocampal CA3 neurons (Weiner et al., 1999). The current study sought to evaluate the effect of ethanol on kainate receptor-mediated inhibition of eIPSCs in rat hippocampal CA1 pyramidal cells. Consistent with previous studies, we found that activation of kainate receptors by 1 μM kainate significantly inhibited eIPSCs recorded from rat hippocampal CA1 pyramidal neurons. This inhibition involved the indirect activation of presynaptic $GABA_B$ receptors, because pretreating slices with a $GABA_B$ receptor antagonist blocked the inhibitory effect of kainate on eIPSCs. Pretreating slices with ethanol, at concentrations as low as 20 mM, significantly reduced kainate inhibition of eIPSCs. In contrast, ethanol did not antagonize the depressant effect of a $GABA_B$ receptor agonist on eIPSCs. Taken together, these results demonstrate that, in addition to its inhibitory effect on postsynaptic kainate receptors in CA3 neurons, relatively modest concentrations of ethanol also significantly antagonize kainate receptor-mediated inhibition of GABAergic synaptic transmission in the CA1 region of the rat hippocampus.

Ethanol Inhibition of Interneuronal Kainate Receptor Function. In this study, bath application of ethanol significantly potentiated eIPSCs evoked by proximal stimulation, as we (Weiner et al., 1997) and others (Poelchen et al., 2000) have reported previously. This effect was primarily on the area of eIPSCs and was significant at 40 and 80 mM ethanol. Bath application of 1 μM kainate inhibited eIPSCs in the presence of ethanol; however, the magnitude of this inhibition was significantly reduced at all but the lowest ethanol concentration tested (10 mM). Thus, ethanol antagonism of kainate inhibition of eIPSCs seemed to be more potent than its direct potentiating effect on eIPSCs. Moreover, the potency of ethanol's depressant effect on kainate inhibition of eIPSCs was the same as that of ethanol antagonism of kainate EPSCs in CA3 pyramidal cells (Weiner et al., 1999). These data suggest that ethanol's overall facilitatory effect on proximal GABAergic synapses may be even more potent under physiological conditions in which presynaptic kainate receptors are active. Although we did not observe any regulatory effect of presynaptic kainate receptors on eIPSCs in the absence of exogenous kainate application in this study, synaptically released glutamate has been shown to modulate GABAergic synaptic transmission via activation of kainate receptors in other studies (Min et al., 1999; Jiang et al., 2001). In general, the synaptic activation of kainate receptors is most readily observed after intense or high-frequency stimulation of glutamatergic afferents (for review, see Frerking and Nicoll, 2000). Therefore, when glutamatergic synaptic transmission is increased, for example during chronic ethanol withdrawal (Tsai and Coyle, 1998), ethanol inhibition of presynaptic kainate receptor function at GABAergic synapses may serve to further enhance the depressant effects of this drug on hippocampal function. Interestingly, there is some evidence that ethanol potentiation of eIPSCs is enhanced after chronic intermittent ethanol exposure (Kang et al., 1998).

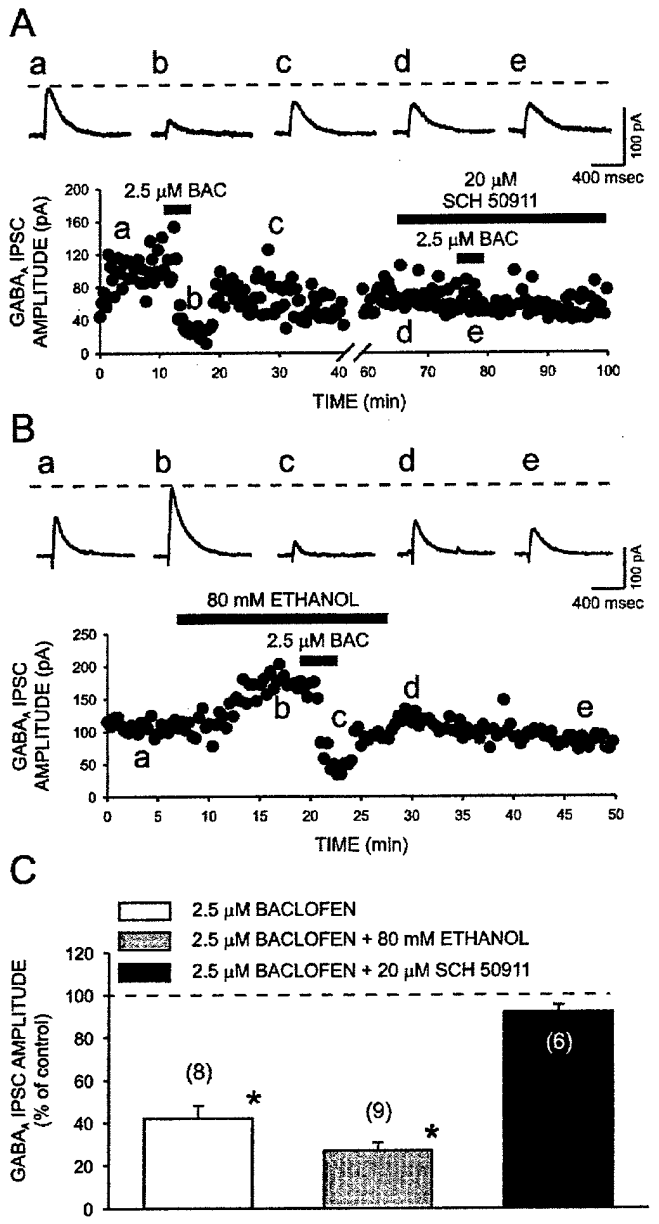

Fig. 5. Ethanol does not antagonize kainate inhibition of $GABA_A$ IPSCs via an indirect effect on presynaptic $GABA_B$ receptors. A, time course of the effect of the $GABA_B$ receptor agonist baclofen (BAC) on the amplitude of $GABA_A$ IPSCs in the absence and presence of SCH 50911. Note that BAC inhibits the amplitude of $GABA_A$ IPSCs and this effect is blocked by 20 μM SCH 50911. B, time course illustrating the effect of 80 mM ethanol pretreatment on BAC inhibition of $GABA_A$ IPSCs. Note that ethanol potentiates the amplitude and area of $GABA_A$ IPSCs but does not occlude the inhibitory effect of BAC. C, bar graph summarizing the effect of bath application of 2.5 μM BAC on the amplitude of $GABA_A$ IPSCs alone, or after pretreatment with 80 mM ethanol or 20 μM SCH50911. $\star$, $P < 0.05$, relative to control.

As previously shown (Frerking et al., 1999), kainate receptor-dependent inhibition of eIPSCs at interneuron-CA1 pyramidal cell synapses in the current study seemed to be largely due to the secondary activation of presynaptic $GABA_B$ receptors. It was therefore necessary to determine whether ethanol was interacting with interneuronal kainate receptor function or rather with the presynaptic $GABA_B$ receptors that are known to depress GABA release (Davies et al., 1990). We therefore tested whether ethanol had any effect on inhibition of eIPSCs mediated by the direct activation of presynaptic $GABA_B$ receptors. Under our recording conditions, 2.5 μM baclofen inhibited eIPSCs to a similar extent as 1 μM kainate. Pretreating slices with the highest concentration of ethanol tested in this study (80 mM) did not inhibit the effect of baclofen on eIPSCs. In fact, ethanol had a modest but significant facilitatory effect on baclofen inhibition of eIPSCs and this novel interaction is currently under further investigation in our laboratory. Therefore, the inhibitory effect of ethanol on kainate inhibition of eIPSCs could not be attributed to an interaction between ethanol and presynaptic $GABA_B$ receptors.

Postsynaptic shunting has also been shown to contribute significantly to the inhibition of eIPSCs by kainate receptor activation at interneuron-CA1 pyramidal cell synapses (Frerking et al., 1999). However, postsynaptic shunting did not seem to contribute to the inhibitory effect of kainate on eIPSCs in this study because bath application of 1 μM kainate was not associated with any changes in input resistance or holding current. It should be noted that postsynaptic shunting of eIPSCs observed in the previous study was demonstrated with a kainate concentration 10 times higher than that used in the present study. Lower concentrations of kainate have previously been reported to have only minimal effects on the passive membrane properties of CA1 pyramidal cells (Bureau et al., 1999).

Taken together, these results further suggest that ethanol may interact directly with interneuronal kainate receptors, in a manner similar to its inhibitory effect on postsynaptic kainate receptors on CA3 pyramidal cells.

Ethanol Sensitivity of Non-NMDA Receptors. Our data suggest that hippocampal kainate receptors may be particularly sensitive to low concentrations of ethanol. These findings are somewhat surprising because few studies have demonstrated ethanol sensitive non-NMDA receptors in neuronal preparations (Martin et al., 1991; Nie et al., 1994). In the current study, as well as in another recent study from our laboratory (Weiner et al., 1999), ethanol was shown to potently inhibit pre- and postsynaptic kainate receptor function, but not AMPA receptor function, in the rat hippocampus. It might then be hypothesized that the ethanol sensitivity of non-NMDA receptors is dependent on the receptor subtype (i.e., kainate versus AMPA) and/or on the subunit composition of these receptors. However, studies conducted with recombinant kainate receptors or native receptors in cultured cells suggest that this is unlikely. For example, Valenzuela and Cardoso (1999) demonstrated that the ethanol sensitivity of recombinant kainate receptors, unlike that of NMDA and $GABA_A$ receptors (Masood et al., 1994; Harris et al., 1997), does not vary with the particular subunits being expressed. Second, recombinant AMPA receptors expressed in either *Xenopus* oocytes (Dildy-Mayfield and Harris, 1992) or human embryonic kidney 293 cells (Lovinger 1993), as well as AMPA receptors in primary culture (Wirkner et al., 2000) are potently inhibited by ethanol. Finally, studies conducted in cultured neurons have reported that ethanol inhibits both kainate and AMPA receptors, with little difference in the potency of these effects (Valenzuela et al., 1998a).

The factors responsible for the differential ethanol sensitivity of native non-NMDA receptors in tissue slices, native receptors in cultured cells, and recombinant receptors in

expression systems are not known. One hypothesis is that receptors in these different environments might undergo differences in post-translational modifications such as phosphorylation, glycosylation, or protein-protein interactions that might alter the ethanol sensitivity of these receptors. Post-translational modifications have been shown to underlie changes in the ethanol sensitivity of NMDA receptors (for review, see Chandler et al., 1998). For example, phosphorylation reduces the ethanol sensitivity of these receptors during acute tolerance (Miyakawa et al., 1997). Although the ethanol sensitivity of kainate receptors seems to be unaltered by phosphorylation (Valenzuela et al., 1998b), the effect of phosphorylation on the ethanol sensitivity of AMPA receptors has not been examined. Post-translational modifications have been shown to account for other differences in the physiological and pharmacological properties of native and recombinant glutamate receptors (Standley and Baudry, 2000). For example, modulation of glutamate receptor function by concanavalin A has been shown to require glycosylation (Everts et al., 1997). Differences in the post-translational regulation of glutamate receptors in brain slices, cultured cells, and expression systems could contribute to previously observed differences in the ethanol sensitivity of these receptors. Clearly, further studies are needed to resolve the physiological mechanisms underlying the differential ethanol sensitivity of native and recombinant glutamate receptors.

Possible Behavioral Significance of Ethanol Inhibition of Interneuronal Kainate Receptors. Our data suggest that, at concentrations relevant to the pharmacological effects of ethanol, this drug may inhibit the activity of at least two populations of kainate receptors in the rat hippocampus. Assessing the behavioral significance of these observations at present is difficult because the physiological role of kainate receptors in this brain region is complex and not fully defined. For example, although kainate clearly inhibits eIPSCs, it likely does so via a profound excitation of presynaptic GABAergic interneurons and an associated increase in spontaneous GABA release. Moreover, recent data suggests that kainate may actually increase unitary eIPSCs under some conditions (Jiang et al., 2001). A further complication is that presynaptic kainate receptors also regulate glutamate release in the CA3 and CA1 regions and the ethanol sensitivity of these receptors remains to be determined. Nevertheless, studies with systemic administration or local infusion of kainate into the hippocampus clearly indicate that the overall effect of kainate receptor activation on hippocampal physiology is profoundly excitatory in nature (Ben-Ari and Cossart, 2000). Therefore, it is likely that acute inhibitory effects of ethanol on hippocampal kainate receptor function will have a predominantly depressant effect on CNS activity, consistent with the known physiological sequelae associated with ethanol ingestion. Continued research into the physiological role of kainate receptors in the mammalian CNS will ultimately allow us to place the effects of ethanol on kainate receptor function into their proper context.

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Alcohol potently inhibits the kainate receptor-dependent excitatory drive of hippocampal interneurons

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Kainate receptors (KA-Rs) are members of the glutamate-gated family of ionotropic receptors, which also includes *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors. KA-Rs are important modulators of interneuron excitability in the CA1 region of the hippocampus. Activation of these receptors enhances interneuron firing, which robustly increases spontaneous inhibitory postsynaptic currents in pyramidal neurons. We report here that ethanol (EtOH) potently inhibits this KA-R-mediated effect at concentrations as low as those that can be achieved in blood after the ingestion of just 1–2 drinks (5–10 mM). Pressure application of kainate, in the presence of AMPA and NMDA receptor antagonists, evoked depolarizing responses in interneurons that triggered repetitive action potential firing. EtOH potently inhibited these responses to a degree that was sufficient to abolish action potential firing. This effect appears to be specific for KA-Rs, as EtOH did not affect action potential firing triggered by AMPA receptor-mediated depolarizing responses. Importantly, EtOH inhibited interneuron action potential firing in response to KA-R activation by synaptically released glutamate, suggesting that our findings are physiologically relevant. KA-R-dependent modulation of glutamate release onto pyramidal neurons was not affected by EtOH. Thus, EtOH increases excitability of pyramidal neurons indirectly by inhibiting the KA-R-dependent drive of γ-aminobutyric acid (GABA)ergic interneurons. We postulate that this effect may explain, in part, some of the paradoxical excitatory actions of this widely abused substance. The excitatory actions of EtOH may be perceived as positive by some individuals, which could contribute to the development of alcoholism.

Kainate receptors (KA-Rs) are glutamate-gated ion channels that play important roles in the regulation of hippocampal excitability. In mossy fiber-to-CA3 pyramidal neuron synapses, KA-Rs mediate synaptic currents and plasticity and modulate glutamate release presynaptically (reviewed in refs. 1 and 2). Although KA-Rs are also present in CA1 pyramidal neurons, these receptors are not activated synaptically (3, 4). In the CA1 region, however, KA-Rs inhibit glutamate release presynaptically (5–7) and also regulate action potential (AP)-dependent GABA release from interneurons; studies have shown that the frequency of AP-independent GABA release is either unaffected (8–10) or inhibited by KA-R activation (11). Activation of KA-Rs by micromolar concentrations of KA inhibits evoked γ-aminobutyric acid type A ($GABA_A$) receptor ($GABA_A$-R)-mediated inhibitory postsynaptic currents (eIPSCs) in CA1 pyramidal neurons (8, 9, 12–15). However, the precise mechanism and the physiological importance of this effect are a matter of controversy (reviewed in refs. 1 and 16). This effect was initially interpreted to indicate that activation of interneuronal KA-Rs exerts a disinhibitory effect on CA1 pyramidal neurons, but subsequent studies have challenged this interpretation. Studies from different laboratories have consistently shown that KA-Rs depolarize interneurons and induce repetitive AP firing, which results in a robust increase in the frequency of $GABA_A$-R-mediated spontaneous IPSCs (sIPSCs) in CA1 pyramidal neurons (4, 8, 9). Importantly, activation of KA-Rs by synaptically released glutamate enhances axonal excitability in interneurons and increases sIPSC frequency in CA1 pyramidal neurons (10, 17). Therefore, the KA-R-mediated increase of interneuronal GABA release may function as an important homeostatic mechanism that prevents overexcitation of CA1 pyramidal neurons (1, 10, 18). Indeed, it was recently demonstrated that activation of GluR5-containing KA-Rs in interneurons inhibits CA1 pyramidal neurons and prevents seizure propagation in the neonatal hippocampus (19).

KA-Rs have emerged as important targets of alcohol's actions in the central nervous system. Ethanol (EtOH) inhibits recombinant KA-Rs in nonneuronal expression systems and native KA-Rs in cultured neurons at concentrations ≥25 mM (legal intoxication limit in the U.S. is 0.08 g/dl = 17 mM) (20–23). KA-R-mediated synaptic currents in CA3 pyramidal neurons in hippocampal slices are also inhibited by ≥20 mM EtOH (24). Moreover, it was recently discovered that 20–80 mM EtOH inhibits the KA-R-mediated inhibition of eIPSCs in CA1 pyramidal neurons (15), suggesting that KA-Rs in interneurons may also be sensitive to pharmacologically relevant concentrations of this drug of abuse.

Here, we provide direct evidence of a potent inhibitory effect of EtOH on interneuronal KA-Rs, which mediate a significant portion of the glutamatergic excitatory drive of these neurons (25). We demonstrate that interneuron firing in response to KA-R activation is inhibited by EtOH concentrations that can be achieved in blood after the ingestion of just one to two drinks (5–10 mM). We also show that this effect of EtOH results in a substantial decrease in the frequency of KA-R-driven sIPSCs in pyramidal neurons. We postulate that the effects of EtOH on interneuronal KA-Rs could contribute, at least in part, to some of the paradoxical excitatory actions that can be induced by low doses of this widely abused substance.

Materials and Methods

Unless indicated, all chemicals were from Sigma. Experiments were performed in coronal hippocampal slices that were prepared from 21- to 40-day-old male Sprague–Dawley rats. For recordings of sIPSCs from CA1 pyramidal neurons and all interneuron recordings, rats were anesthetized with ketamine 250 mg/kg, and 350- to 400-μM-thick slices were prepared with a vibratome as described (26). Artificial cerebrospinal fluid (ACSF) contained 126 mM NaCl, 3 mM KCl, 1.25 mM

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; AMPAR, AMPA receptor; AP, action potential; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; EtOH, ethanol; GABA, γ-aminobutyric acid; IPSC, inhibitory postsynaptic current; sIPSC, spontaneous IPSC; eIPSC, evoked IPSC; KA, kainate; KA-R, KA receptor; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor.

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NaH_2PO_4, 1 mM $MgSO_4$, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 0.1 mM DL-AP5 (Tocris–Cookson, Bristol, U.K.), and 10 mM glucose, equilibrated with 95% O_2/5% CO_2. The α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor (AMPAR) blocker, GYKI 53655 (30 μM; custom synthesized by Tocris–Cookson), was added to the ACSF to isolate KA-R-mediated responses. When indicated, bicuculline methiodide, 6,7-dinitroquinoxaline-2,3-dione (DNQX; Alexis, San Diego), SCH-50911 (Tocris–Cookson) or EtOH (AAPER Chemical, Shelbyville, KY) were added to the ACSF. After a recovery time of $\geq$80 min, slices were transferred to a chamber perfused with ACSF at a rate of 2–3 ml/min. Whole-cell patch-clamp electrophysiological recordings from CA1 pyramidal neurons and stratum radiatum–lacunosum moleculare interneurons were performed under infrared-differential interference contrast microscopy at 34°C with an Axopatch 200B amplifier (Axon Laboratories, Union City, CA). Interneurons were identified on the basis of their morphological characteristics, as described elsewhere (27); i.e., their somata appeared round or ovoid and they lacked discernible thick bifurcating apical dendritic processes. We confirmed that neurons with these characteristics corresponded to interneurons by passively filling some of them with 0.5% biocytin followed by fixation in 4% paraformaldehyde and staining with 0.1% streptavidin-cy3 (Jackson Immunolaboratories, West Grove, PA) as described elsewhere (28). Interneurons were visualized with a LSM-510 confocal microscope (Carl Zeiss, Thornwood, NY; University of New Mexico Cancer Center) and their neuronal arborizations were reconstructed from z axis projection images by using LSM5 image browser software. Microelectrodes had resistances of 3–5 MΩ. We recorded sIPSCs at a holding potential of -60 mV by using internal solution containing 140 mM CsCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, 10 mM Hepes (pH 7.3), 2 mM Na_2-ATP, and 2 mM QX-314 (Tocris–Cookson). The effect of KA-R activation on sIPSC frequency ran down after sequential applications of KA. Therefore, the effect of EtOH on the KA-R-mediated increase of sIPSC frequency was assessed on neurons from slices that had not been previously exposed to KA. Current-clamp experiments ($I_{holding}$ = 0, unless indicated) were performed with an internal solution containing 135 mM K-gluconate, 10 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 mM EGTA, 10 mM Hepes (pH 7.3), and 2 mM Na_2-ATP. Access resistances were between 20 and 35 MΩ; if access resistance changed >20%, the recording was discarded. A pneumatic picopump (World Precision Instruments, Sarasota, FL) was used to apply puffs of KA or AMPA (Tocris–Cookson). The puffing pipette was placed $\approx$200 μm from the cell, the pressure was varied between 4 and 7 psi, and the duration was between 2 and 10 sec. Trains of excitatory postsynaptic potentials (EPSPs) were evoked with a concentric bipolar electrode (FHC, Bowdoinham, ME) placed in the stratum radiatum. Data were acquired and analyzed with pClamp7 or 8 (Axon Laboratories); sIPSCs were analyzed with MINIS ANALYSIS program (Synaptosoft, Decatur, GA). For the experiments on the presynaptic actions of KA on glutamatergic terminals, slice preparation and recording methods were as described (24). Data are presented as mean ± SEM.

Results

We studied the modulation by KA of GABAergic tone in CA1 pyramidal neurons in rat hippocampal slices. We recorded IPSCs triggered in pyramidal neurons by the spontaneous AP-dependent release of GABA from interneurons; whole-cell voltage-clamp recordings were performed in the presence of GYKI 53655 (30 μM) and DL-AP5 (100 μM) to block AMPA and *N*-methyl-D-aspartate (NMDA) receptors (NMDAR), respectively. Spontaneous IPSCs were blocked by bicuculline methiodide, indicating that they were mediated by $GABA_A$Rs (Fig. 1*A*; similar results seen in two additional neurons). Application of 0.5 μM KA induced a robust and reversible increase in sIPSC frequency (Figs. 1 *B* and *C* and 2). Ethanol potently inhibited this effect of KA (Figs. 1 *D* and *E* and 2). The KA-R-mediated increase in sIPSC frequency was blocked by the non-NMDA receptor antagonist DNQX (80 μM; n = 3; data not shown). Because GYKI 53655 was present, this finding confirms that the effect of KA was mediated by KA-Rs. Nonlinear regression analysis yielded an EtOH IC_{50} of 4.6 mM (95% confidence interval 2.1–10.1 mM; Fig. 2 *Inset*). Application of EtOH (2–25 mM) alone did not significantly affect basal sIPSC frequency (Figs. 1 *D* and *E* and 2); 50 mM EtOH induced a small

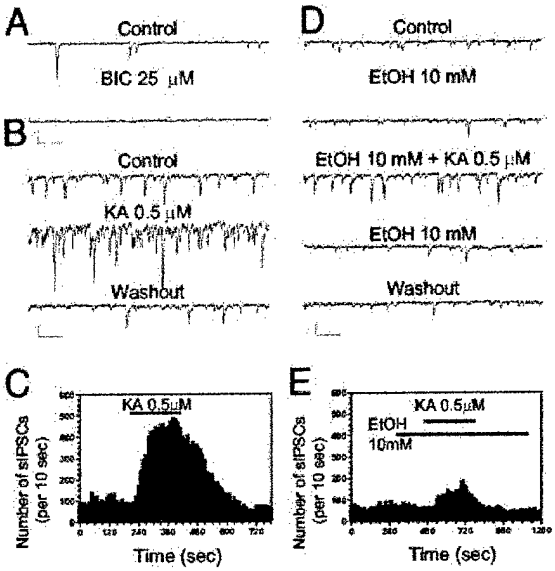

Fig. 1. EtOH inhibits the KA-R-mediated increase of sIPSC frequency in CA1 pyramidal neurons. (*A*) sIPSCs are fully blocked by the $GABA_A$-R antagonist, bicuculline (BIC; 25 μM). (*B* and *C*) Bath application of KA (0.5 μM) for 3.5 min, in the presence of GYKI 53665 (30 μM) and DL-AP5 (100 μM), induced a robust and reversible sIPSC frequency increase. (*D* and *E*) EtOH (10 mM) did not affect either the basal amplitude or frequency of sIPSCs, but reduced the increase in frequency induced by KA-R-activation. (Scale bars: 100 pA and 250 ms.)

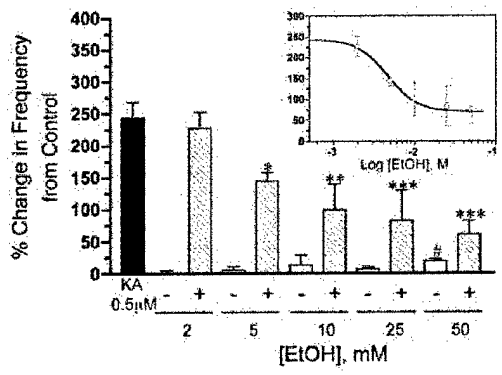

Fig. 2. EtOH inhibits the KA-R-mediated effect on sIPSC frequency in a concentration-dependent manner. The filled bar represents the average percent change induced by KA (0.5 μM) on sIPSC frequency. Striped bars represent the effect of increasing concentrations of EtOH on this KA-R-dependent effect. Open bars represent the percent change in basal sIPSC frequency induced by EtOH alone. (*Inset*) A nonlinear regression fit of the inhibitory effect of EtOH on the KA-R-induced increase of sIPSC frequency (IC_{50} = 4.6 mM). Each bar or symbol represents the mean ± SEM of 7–28 neurons. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, by one-way ANOVA followed by Bonferroni's multiple comparison test; #, $P < 0.05$, by one sample t test versus theoretical mean of zero.

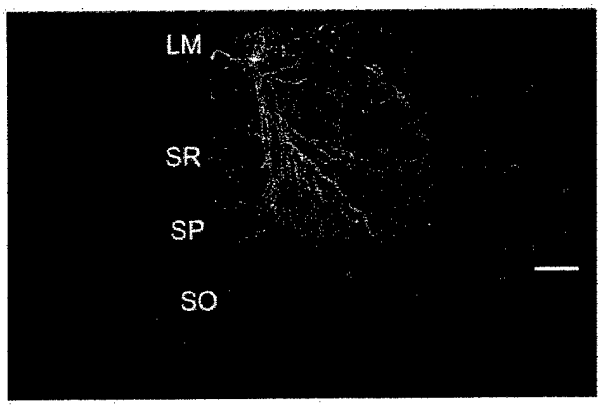

Fig. 3. Anatomical reconstruction of a stratum radiatum-stratum lacunosum moleculare interneuron. Shown is a single *z* axis projection of 20 confocal microscopy sections (4 μm) of one of the interneurons that were studied electrophysiologically. See *Materials and Methods* for details of histochemical procedures. Similar results were obtained with six additional neurons (data not shown). (Scale bar: 100 μm.) L-M, stratum lacunosum moleculare; SR, stratum radiatum; SP, stratum pyramidale; SO, stratum oriens.

but significant increase in sIPSC frequency (Fig. 2). The average sIPSC amplitudes in the presence of 2, 5, 10, 25, and 50 mM EtOH were 4.9 ± 3.7% (n = 10), 1.9 ± 3.2% (n = 8), 3.1 ± 3.5% (n = 7), −0.5 ± 6.9% (n = 7), and 0.9 ± 2.6% (n = 9) of control, respectively (data not shown; see Fig. 1*D* for an illustration of the lack of an effect of 10 mM EtOH alone on sIPSC amplitude).

We also recorded directly from interneurons under whole-cell current-clamp conditions. We studied interneurons located in the stratum radiatum near the stratum lacunosum moleculare, such as the one illustrated in Fig. 3. These interneurons extended neurites mainly into the stratum radiatum, stratum pyramidale, and/or stratum oriens. Pressure application of KA (5 μM in the micropipette located ≈200 μm from the soma) in the presence of GYKI 53655 (30 μM) and DL-AP5 (100 μM), caused a reversible depolarization (14 ± 2 mV; n = 8) and repetitive AP firing (42 ± 7 APs per evoked response; n = 8) in these neurons (Fig. 4). Pressure application of 5 μM AMPA in the absence of GYKI, reversibly depolarized the interneurons to a similar extent (21 ± 3 mV; n = 7) and also caused them to fire repetitive APs (95 ± 15 APs per evoked response; n = 7). Injection of depolarizing current pulses (35 pA; 200 msec; V_m = −70 mV) induced a 13 ± 3 mV depolarization in these type of interneurons and repetitive AP firing (6 ± 1 APs per evoked response; n = 5; Fig. 4). Bath application of EtOH (10 mM) significantly reduced the amplitude of KA-R-mediated evoked potentials sufficiently to abolish AP firing (Fig. 4). In contrast, EtOH did not significantly affect the amplitude of AMPAR-mediated evoked potentials or the AP firing in response to AMPA (Fig. 4). Moreover, it did not affect the amplitude of responses evoked by depolarizing current injection or AP firing in response to this depolarization (Fig. 4). Application of 10 mM EtOH alone did not significantly affect either the interneuronal resting membrane potential (control = −68 ± 3 mV and EtOH = −69 ± 3 mV; n = 20) or the membrane resistance (control = 302 ± 17 MΩ and EtOH = 277 ± 15 MΩ; n = 5).

We next assessed the effect of EtOH on KA-R-mediated interneuron EPSPs evoked by synaptic glutamate release from Schaffer collateral axonal terminals. These experiments were also performed in the whole-cell current-clamp mode. EPSPs were evoked by trains of five stimuli at 20 Hz to maximize the induction of AP firing. In the presence of blockers of NMDA (DL-AP5; 100 μM), $GABA_A$ (bicuculline methiodide; 25 μM) and $GABA_B$ (SCH-50911; 20 μM) receptors, repetitive stimulation of the Schaffer collateral reproducibly induced non-

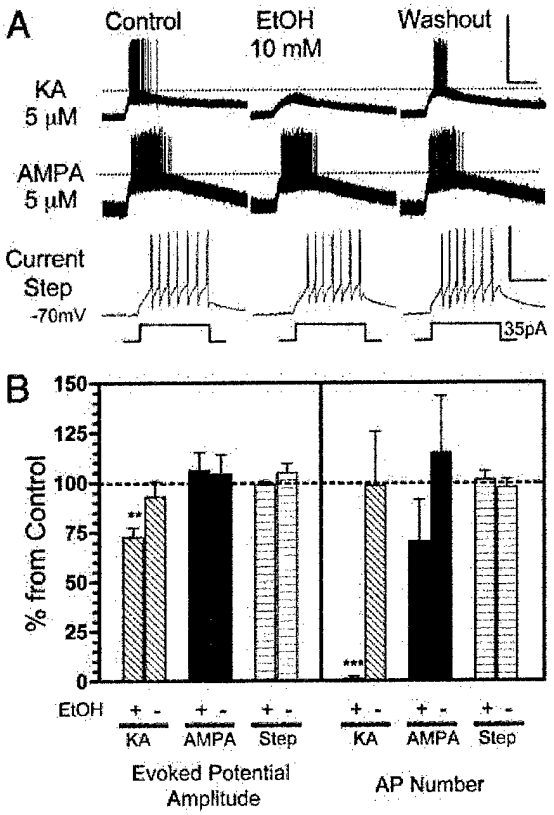

Fig. 4. EtOH inhibits evoked potentials and AP firing triggered by pressure application of KA onto interneurons. (*A*) Sample traces of current–clamp recordings (I = 0) from CA1 stratum radiatum-stratum lacunosum moleculare interneurons. (*Top*) In the presence of DL-AP5 (100 μM) and GYKI 53655 (30 μM), pressure application of KA (5 μM in micropipette located ≈200 μm from the soma) induced reproducible evoked potentials and bursts of AP firing. Bath application of EtOH (10 mM) induced a reversible decrease of the KA-R-mediated evoked potentials and also abolished firing. (*Middle*) The same experiment was performed by pressure-delivering 5 μM AMPA to another interneuron in the presence of DL-AP5 only. EtOH did not inhibit AMPAR-mediated evoked potential amplitude and AP firing. (*Bottom*) Lack of an effect of EtOH on depolarization-induced AP firing in another interneuron. (Scale bars for *Top* and *Middle*: 50 mV and 5 sec; for *Bottom*: 50 mV and 100 msec.) (*B*) Summary of the effects of EtOH on KA-R-dependent and AMPAR-dependent evoked potential amplitude and AP number. Also shown is the summary of the effect of EtOH on the amplitude of depolarization induced by current injection and AP number in response to this current injection. Data were normalized with respect to control responses (represented by the dashed line). +, the effect of 10 mM EtOH; −, the effect of the washout. Each bar represents the mean ± SEM of five to eight neurons. **, $P < 0.01$, ***, $P < 0.001$, by one sample t test versus theoretical mean of 100.

NMDA receptor-mediated EPSPs (peak amplitude: 29 ± 2 mV; n = 4) that triggered AP firing (Fig. 5*A*). In agreement with a recent report (29), GYKI 53655 (30 μM) reduced the peak amplitude of the first non-NMDA EPSP by 63 ± 5% (n = 4) and abolished AP firing (Fig. 5 *A–C*). In the continuous presence of GYKI, an increase in stimulation intensity enhanced EPSP amplitude and restored AP firing (Fig. 5 *A–C*). A subsequent application of EtOH (10 mM) significantly reduced the peak amplitude of the KA-R-mediated compound EPSPs and reduced AP firing (Fig. 5). These events were abolished by DNQX (Fig. 5 *A–C*). Paired-pulse facilitation of KA-R-mediated EPSPs was not affected by EtOH; the ratio of the amplitude of the first to the second EPSP were 2 ± 0.4 (n = 4) and 2.5 ± 0.8 (n = 4) in the absence and presence of EtOH, respectively.

Finally, we determined the effect of EtOH on presynaptic KA-R-dependent modulation of glutamate release in Schaffer

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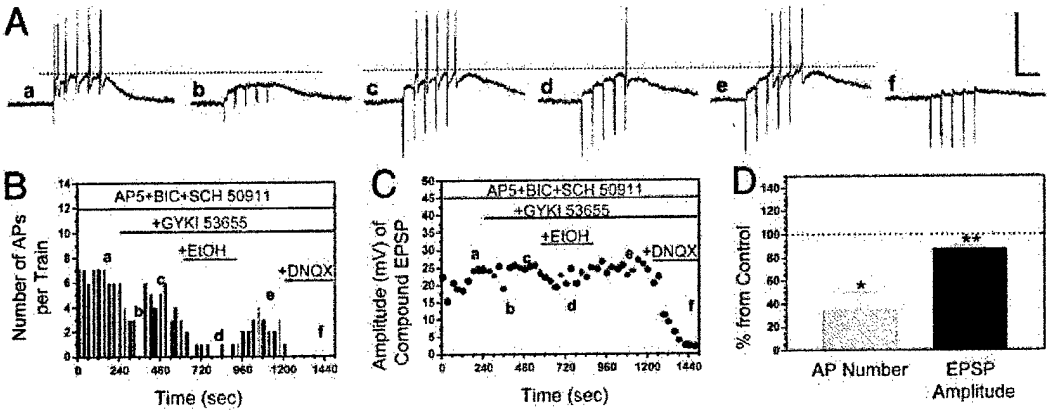

Fig. 5. EtOH inhibits KA-R-mediated interneuron EPSPs and AP firing evoked by stimulation of the Schaffer collaterals. (*A*) Sample traces of current–clamp recordings ($I = 0$) from a CA1 stratum radiatum-stratum lacunosum moleculare interneuron. (*a*) In the presence of DL-AP5 (100 μM), bicuculline (25 μM), and SCH-50911 (20 μM), a train of five stimuli (20 Hz) delivered in the stratum radiatum induced non-NMDA EPSPs that triggered a burst of APs. (*b*) GYKI 53665 (30 μM) blocked the AMPA component of the EPSPs and eliminated firing. (*c*) An elevation in the stimulation intensity increased the amplitude of the KA-R-mediated EPSPs and restored AP firing. (*d* and *e*) Bath application of EtOH (10 mM) reversibly decreased the peak amplitude of the KA-R-mediated compound EPSPs and reduced AP number. (*f*) The KA-R-mediated EPSPs were fully blocked by DNQX (80 μM). (Scale bars: 50 mV and 100 msec.) (*B* and *C*) Time courses illustrating the effects of the sequential application of GYKI, EtOH, and DNQX on EPSP amplitude and AP number in the same cell illustrated in *A*. (*D*) Summary of the effect of EtOH (10 mM) on AP number and EPSP amplitude. Each bar represents the mean ± SEM of five neurons. *, $P < 0.05$; **, $P < 0.01$, by one sample *t* test versus theoretical mean of 100. Data were normalized with respect to control responses (represented by the dashed line).

collateral-to-CA1 pyramidal neuron synapses. We recorded AMPAR-mediated excitatory postsynaptic currents (EPSCs) that were evoked by electrical stimulation of the stratum radiatum in the presence of D-AP5 (50 μM) and bicuculline methiodide (20 μM). In agreement with previous reports (5–7), we found that 1 μM KA inhibited the amplitude of AMPA EPSCs (Fig. 6 *A* and *C*). EtOH (40 and 80 mM) alone had no effect on the amplitude of AMPA EPSCs and it did not significantly affect the KA-induced inhibition of AMPAR-mediated EPSCs (Fig. 6 *B* and *C*). A comparable result was obtained for NMDAR-mediated EPSCs recorded in the presence of bicuculline methiodide (20 μM) and NBQX (1 μM). NMDAR-dependent EPSCs were inhibited by 46 ± 4% ($n = 17$) and 53 ± 4% ($n = 11$) by 1 μM KA in the absence and presence of 80 mM EtOH, respectively (data not shown).

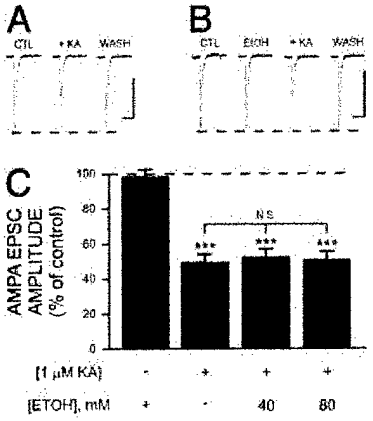

Fig. 6. EtOH has no effect on presynaptic KA receptor-dependent inhibition of AMPA EPSCs in CA1 pyramidal neurons. Traces are averages of five to nine AMPA EPSCs recorded in the presence of bicuculline (20 μM) and D-AP5 (50 μM). Representative traces illustrating the inhibitory effect of 1 μM KA on AMPAR-mediated EPSCs in the absence (*A*) and presence (*B*) of 80 mM EtOH. Note that EtOH alone has no effect on the amplitude of AMPA EPSCs or KA inhibition of AMPA EPSCs. (Scale bars: 100 pA and 200 msec.) (*C*) Summary of the effect of EtOH on KA inhibition of AMPA EPSCs. ***, $P < 0.001$ with respect to responses obtained in the presence of EtOH alone by one-way ANOVA; each bar represents the mean ± SEM of 7–27 neurons. Data were normalized with respect to control responses (represented by the dashed line).

Discussion

We demonstrate here that EtOH potently inhibits KA-R function in CA1 hippocampal interneurons. We initially tested the effect of EtOH on KA-R-mediated modulation of GABAergic tone in this region. In agreement with previous reports, we found that bath application of KA, in the presence of AMPAR and NMDAR blockers, enhances spontaneous AP-dependent GABA release from interneurons, resulting in a massive increase in sIPSC frequency in pyramidal neurons (8, 9, 18). Importantly, we found that EtOH inhibits this KA-R-dependent effect with an IC_{50} of 4.6 mM. By comparison, a number of studies have reported that EtOH concentrations ≥10 mM are required to significantly inhibit NMDAR-dependent population EPSPs in the CA1 region of hippocampal slices (30–32). Moreover, significant inhibition of AMPAR-mediated population EPSPs was only observed with 100 mM EtOH in this hippocampal region (30). This result is in agreement with our finding that AMPAR-mediated responses in CA1 pyramidal neurons and interneurons are not significantly affected by EtOH. Consequently, interneuronal KA-Rs appear to be the most EtOH sensitive glutamatergic ionotropic receptor subtype in the CA1 region of the rat hippocampus. This conclusion can be extended to the CA3 hippocampal region, where we found that KA-Rs are the only members of the ionotropic glutamate receptor family that are significantly inhibited by 20–40 mM EtOH (24).

Our study also demonstrates that 2–50 mM EtOH does not affect the amplitude of basal $GABA_A$-mediated sIPSCs. This result is in general agreement with several reports that concentrations of EtOH ≥40 mM are required to significantly potentiate $GABA_A$-R-mediated postsynaptic responses in CA1 pyramidal neurons (refs. 33 and 34; reviewed in ref. 35). Moreover, we found that EtOH, at concentrations ≤25 mM, does not significantly affect basal sIPSC frequency, indicating that it does not directly modulate GABA release in response to spontaneous AP firing in CA1 interneurons. It also suggests that spontaneous GABAergic transmission is not under the tonic control of KA-Rs under our recording conditions. If this had been the case, we would have expected to observe inhibition of basal sIPSC

frequency in the presence of EtOH. This finding is not completely unexpected given that glutamate is just one of the many neurotransmitters that regulate spontaneous firing in CA1 hippocampal interneurons (36). Indeed, several studies have demonstrated that blockade of glutamate receptors does not significantly inhibit basal sIPSC frequency in hippocampal pyramidal neurons (37, 38). It should be noted that KA-Rs may control spontaneous CA1 interneuronal firing under some conditions; it was recently established that KA-R-mediated spontaneous EPSCs can contribute ≈50% of the glutamate-induced current in CA1 interneurons in slices from immature rats (25).

Crowder *et al.* (15) recently found that EtOH, at concentrations ≥20 mM, significantly inhibits the KA-R-dependent inhibition of eIPSCs in CA1 pyramidal neurons. In contrast, the results of the present study indicate that EtOH concentrations as low as 5 mM are sufficient to inhibit the KA-R-mediated increase in sIPSC frequency in these neurons. The apparent differential EtOH sensitivity of the KA-R-mediated effects described by Crowder *et al.* (15) and those presented here may simply reflect that assessing the effect of EtOH on the KA-R-dependent increase in sIPSC frequency is a more sensitive assay. Alternatively, this discrepancy could be caused by the fact that two different populations of KA-Rs may mediate the inhibition of eIPSCs and the potentiation of sIPSC frequency (11). For instance, the KA-induced inhibition of monosynaptic IPSCs, but not the potentiation of sIPSCs, has been shown to depend on the activation of $G_{i/o}$ proteins, phospholipase C, and protein kinase C in the presynaptic terminal (11). However, the KA-R-induced increase in the success rate of unitary IPSCs is not affected by protein kinase C inhibition (10). In addition, activation of KA-Rs with low concentrations of glutamate causes a significant reduction in eIPSC amplitude without affecting sIPSC frequency (11). Thus, it is possible that KA-Rs that inhibit eIPSCs have lower sensitivity to EtOH than those that potentiate sIPSCs, which could be due to differences in their subunit composition, posttranslational regulation, or association with other proteins. The latter possibility seems more likely given that recombinant KA-Rs with specific differences in their subunit composition are all similarly sensitive to acute EtOH exposure (21) and that changes in phosphorylation of homomeric recombinant GluR6 receptors do not affect acute sensitivity to EtOH (39). However, it must be kept in mind that the role of subunit composition and phosphorylation in determining the sensitivity of native KA-Rs to EtOH has yet to be investigated. For instance, studies with knockout mice lacking GluR5 and GluR6 subunits indicate that KA-Rs in interneurons of the stratum radiatum are heteromers containing both of these subunits (18) and their coassembly may increase sensitivity to acute EtOH. As for KA-Rs in GABAergic interneurons, differences in subunit composition, association with other proteins or posttranslational regulation could also explain the lack of sensitivity to EtOH of KA-Rs in CA1 glutamatergic terminals. For instance, KA-Rs in CA1 glutamatergic terminals modulate excitatory synaptic transmission via a presynaptic G protein-dependent mechanism (7).

Taken together, the results of the study of Crowder *et al.* (15) and those reported here indicate that EtOH can exert apparently opposite actions on the excitability of pyramidal neurons via its interactions with interneuronal KA-Rs. On one hand, it reduces the KA-R-dependent excitatory drive to interneurons, which decreases tonic (i.e., sIPSCs) GABAergic transmission in pyramidal neurons (i.e., disinhibits). On the other, EtOH decreases the KA-R-dependent inhibition of phasic (i.e., eIPSCs) GABAergic transmission in these neurons (i.e., increases inhibition). Thus, the findings of these studies raise the question as to which of these effects would be more physiologically relevant *in vivo*. Given that pyramidal neurons in the intact hippocampus should receive GABAergic input in the form of phasic activity superimposed on tonic activity, we believe that both effects are important. The EtOH-induced decrease in the KA-R-driven GABAergic inhibitory tone will reduce responsiveness of pyramidal neurons to excitatory input. Our data suggest that the dominant effect at very low EtOH concentrations (5–10 mM) will be to depress this inhibitory tone, thereby increasing neuronal excitability. On top of this effect, EtOH will also decrease the KA-R-dependent inhibition of eIPSCs at higher concentrations (i.e., ≥20 mM). Assuming that the physiological counterpart of an eIPSC corresponds to the phasic activity driven by the coordinated firing of interneuronal ensembles, then the EtOH-induced attenuation of the KA-R-mediated inhibition of eIPSCs will likely have an impact on network activity in the CA1 region (40, 41). Indeed, it has been demonstrated that an enhancement in the glutamatergic excitatory drive to pools of hippocampal interneurons increases the frequency of their oscillatory activity (42). However, whether KA-Rs participate in the regulation of this type of activity remains an open question for future research. It should be emphasized, however, that at concentrations ≥20 mM, EtOH will also affect (*i*) KA-Rs in CA3 pyramidal neurons (24) and (*ii*) other neuronal proteins (for example, $GABA_A$-Rs and NMDARs). The end result of its combined actions on all of these proteins is believed to cause neuronal depression (reviewed in ref. 35).

A key finding of our study is that the mechanism of action of EtOH involves an all-or-none effect on interneuronal firing. We found that EtOH inhibited KA-R-mediated evoked potentials to a degree that is sufficient to abolish interneuron firing in response to activation of these receptors. This finding explains the relatively steep relationship between EtOH concentration and inhibition of the KA-induced increase in sIPSC frequency. This steep concentration dependence likely reflects the EtOH-induced progressive inhibition of KA-R-evoked firing in the different interneurons that synapse onto the particular pyramidal neuron under study. Interestingly, 10 mM EtOH inhibited responses evoked by pressure application of KA by only ≈25%; however, the KA-induced increase in sIPSC frequency was reduced by ≈60% by this concentration of EtOH. These findings suggest that a relatively modest effect of ethanol on interneuronal KA-Rs becomes nonlinearly amplified into a more sub-

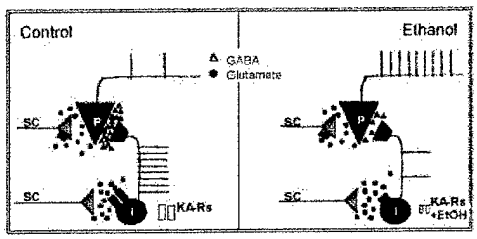

Fig. 7. Simplified model of the EtOH-induced inhibition of the KA-R-mediated excitatory drive of interneurons. (*Left*) Under control conditions, glutamate (circles) released from the Schaffer collaterals (SC) activates interneuronal KA-Rs, which depolarize interneurons (I), and induces AP firing. This effect triggers sIPSCs onto CA1 pyramidal neurons (P); GABA is represented by triangles. Presynaptic KA-Rs in Schaffer collateral glutamatergic terminals that synapse onto CA1 pyramidal neurons are depicted in gray. Activation of these receptors inhibits glutamate release. The net effect of KA-Rs activation is to increase tonic inhibition of CA1 pyramidal neuron's excitability. AMPARs and axonal KA-Rs are not depicted for clarity. (*Right*) In the presence of low concentrations of EtOH (5–10 mM), KA-Rs in interneurons are inhibited, which reduces the excitatory drive to these neurons, reduces $GABA_A$-R-mediated sIPSCs, and increases the excitability of pyramidal neurons. KA-Rs in glutamatergic terminals are unaffected by EtOH. The effects of higher concentrations of EtOH (≥20 mM) are not depicted for clarity (see *Discussion*); at these concentrations, EtOH will (*i*) reduce the inhibitory effect of KA-R activation on eIPSCs (15), (*ii*) inhibit KA-R-mediated synaptic currents in CA3 pyramidal neurons (24), and (*iii*) modulate the function of other neuronal proteins, including $GABA_A$-Rs and NMDARs.

stantial decrease in tonic inhibition of principal neurons. This suggestion is important given that pharmacologically relevant concentrations of EtOH have generally been found to produce relatively small effects on the function of neurotransmitter-gated and voltage-gated ion channels (reviewed in ref. 35). Thus, our results clearly illustrate that effects of EtOH, which may seem minimal when examined in isolated neurons, could have a dramatic impact on the excitability of neurons when studied in the context of a circuit such as the one studied here.

Our study also shows that EtOH inhibits interneuron firing in response to KA-R activation by synaptically released glutamate. This finding suggests that the results obtained with exogenous application of KA have physiological relevance. We postulate that the effects of EtOH on interneuronal KA-Rs are likely to have profound effects on the excitability of pyramidal neurons as illustrated by the simplified model shown in Fig. 7. Numerous studies have established that at concentrations produced by the ingestion of one to two drinks (5–10 mM), EtOH causes paradoxical excitatory effects, including memory facilitation (43–45). Importantly, an increase in the spontaneous firing rate of single units in the dorsal hippocampus has been demonstrated after injection of low doses of EtOH into rats (46). However, mechanisms explaining how a powerful central nervous system depressant such as EtOH can produce these paradoxical excitatory effects have remained elusive. The results of our study suggest that inhibition of the KA-R-dependent excitatory drive of inhibitory interneurons could explain, at least in part, some of these stimulatory actions of EtOH, which may be perceived as positive by some individuals and contribute to the development of alcoholism.

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